

## RAPID GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING *ECLIPTA PROSTRATA* LEAF EXTRACT

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### ABSTRACT

Silver nanoparticles were successfully synthesized from silver nitrate through a rapid green synthesis method using *Eclipta prostrata* leaf extract as a reducing cum stabilizing agent. The experimental procedure was readily conducted at room temperature and pressure, and could be easily scaled up. The silver nanoparticles thus obtained were characterized using UV-Visible Spectroscopy (UV-VIS) which yielded an absorption peak at 416 nm. The biomolecules responsible for capping of the bio-reduced silver nanoparticles synthesized using plant extract were successfully identified through FTIR analysis. It was evinced through Scanning Electron Microscope (SEM), and X-ray diffraction (XRD) analysis that the silver nanoparticles were crystalline in nature and spherical in shape. The average size of the particles obtained using Scherrer's formula was 27.4 nm. The adopted technique for silver nanoparticle synthesis is suitable for large-scale production.

**KEYWORDS:** Silver Nanoparticles, Green Synthesis, Characterization, *Eclipta prostrata*

### INTRODUCTION

The use of biomolecules present in plant extracts to reduce metal ions to nanoparticles in a single-step green synthesis process has been found to be cost effective and eco-benign over the physical and chemical methods. Development of bioinspired material for fabrication of nanoparticles is the cutting edge of research and is a suitable process for large-scale production [1, 2]. Among the living organisms, the plant materials gain more attention in the nanoparticle production [3]. The heavy metal-resistant capability and phytoremediation of plants are the basic concept for the synthesis of nanoparticles. The uptake and conversion of metal salts like  $\text{AgNO}_3$ ,  $\text{Na}_3\text{Ag}(\text{S}_2\text{O}_3)_2$ , and  $\text{Ag}(\text{NH}_3)_2\text{NO}_3$  to metal silver nanoparticles was demonstrated in [4]. Metal-resistant capability of plants motivated researchers to adopt environmentally benign novel green nano-factories for the synthesis of noble nanoparticles. Avoiding the maintenance of microbial cell culture is one of the advantages of plants when compared to the microbes. In addition, the plant-mediated synthesis was found to be rapid, flexible, and suitable process for large-scale nanoparticle production. Plant parts like fruit [5], leaf [3], bark [6], seed [7], and stem [8] extracts are being effectively used in green synthesis. Among nanoparticles, silver nanoparticles have been used enormously due to their potent antibacterial [9], antifungal [10], and antitumor activity [11]. The excellent antimicrobial properties of silver nanoparticles is currently being used widely in food packaging [12], preservation [13], medicine and cosmetics [14, 15, 16]. *Eclipta* leaves mediated synthesis of silver nanoparticles was earlier reported by Anal K. Jha et al., [17].

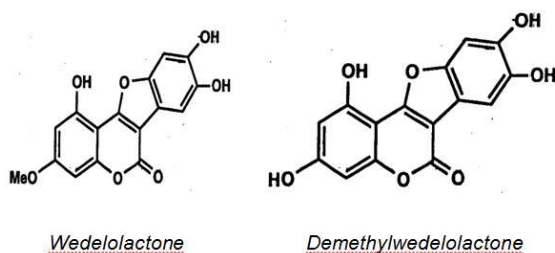
*Eclipta prostrata* (*syn. Eclipta prostrata*) commonly known as **false daisy**, **yerba de tago**, and **bhringraj**, is a species of plant in the family Asteraceae. This plant has cylindrical, grayish roots. The solitary flower heads are 6–8 mm in

diameter, with white florets. The achenes are compressed and narrowly winged. This species grows commonly in moist places as a weed in warm temperate to tropical areas worldwide. Taxonomically authenticated healthy leaves of *Eclipta prostrata* from plants grown in the southern part of India were collected for the synthesis of silver nanoparticles



**Figure 1:** *Eclipta prostrata* (Syn. *Eclipta Alba*, *Eclipta Erecta*, *Verbesina Alba*, *Verbesina Prostrata*) (Image Courtesy: Wikipedia)

The plant has been reported to contain phytosterol,  $\beta$ -amyrin, triterpenes such as ecalbatin, echinocystic acid, ursolic acid, flavones such as Luteolin and coumarin such as wedelolactone and Demethylwedelolactone [18, 19].



**Figure 2:** Structures of Two Important Isolated Phytochemicals from *Eclipta prostrata* [5]

The plant is known to have some important pharmacological activities such as hepatoprotective, antimicrobial, antinociceptive, analgesic, antiinflammatory, antiviral, immunomodulatory and nootropic activity [18].

*E.alba* has been used in traditional systems of medicine and also by traditional healers especially in South region of India for the treatment of epilepsy since ancient times [20]. Reports suggest that Wedelolactone and Luteolin, important constituents of *E.alba* have selectivity and affinity towards Benzodiazepine binding site on GABA receptor [20, 21]. Luteolin also inhibit the release of glutamate at cerebrocortical nerve terminals [23].  $\beta$ -amyrin; a constituent in *E.alba* increases taurine level which is indirectly associated with reduced frequency of epileptic seizure, balance in glutamate levels and neuronal membrane stabilization [24].

Silver nanoparticles have unique optical, electrical, and thermal properties and are being incorporated into products that range from photovoltaics to biological and chemical sensors. Examples include conductive inks, pastes and fillers which utilize silver nanoparticles for their high electrical conductivity, stability, and low sintering temperatures. Additional applications include molecular diagnostics and photonic devices, which take advantage of the novel optical properties of these nanomaterials. An increasingly common application is the use of silver nanoparticles for antimicrobial coatings, and many textiles, keyboards, wound dressings, and biomedical devices now contain silver nanoparticles that

continuously release a low level of silver ions to provide protection against bacteria. Silver has been recognized as having inhibitory effect on microbes [25]. The most important application of silver and silver nanoparticles is in medical industry such as topical ointment to prevent infection against burns and open wounds [26] Silver nanoparticles have been found effective against *E. coli* and *Pseudomonas aeruginosa* [27] *Bacillus cereus*, *Staphylococcus aureus*. The silver nanoparticles synthesized using the *Eclipta prostrata* leaf extract revealed excellent antibacterial activity against different gram classes of bacteria and promises a great application for various pharmaceutical, biomedical, and environmental applications. [28, 29, 30, 31]

Keeping in view the important medical applications of the *Eclipta prostrata* species a single step green synthesis of process of silver nanoparticles using its leaf extract has been pursued and reported successfully.

## **MATERIALS AND METHODS**

### **Plant Material and Preparation of Extract**

Known weight (50g) of freshly collected, taxonomically authenticated healthy leaves of *Eclipta* (Figure 1) were taken and washed thoroughly in flush of tap water in the laboratory for 10 min in order to remove the dust particles and rinsed thoroughly in sterile distilled water. These clean leaves were finely cut and were taken into 250 mL capacity beaker and the contents were stirred in 150 mL double distilled water at 373 K for 15 minutes. The extract was cooled to room temperature, and gently pressed and filtered firstly through sterile serene cloth. This solution was treated as source extract and was utilized in subsequent procedures.

### **Synthesis of Silver Nanoparticles**

1 mM aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. The reaction medium contained 10 ml *Eclipta prostrata* leaf extract and 90 ml of 1mM aqueous solution of silver nitrate. The reaction was continued for 2 h at room temperature. The colour of reaction mixture changed from colourless to brown.

### **UV-Vis Spectra Analysis**

The silver nanoparticles were characterized by UV-Vis spectroscopy. A sample of 1 mL of the suspension was collected after monitoring the completion of bio-reduction of  $\text{Ag}^+$  in aqueous solution, after diluting a small aliquot of the sample with 2 ml of deionized water it was subsequently scanned using an UV-Vis spectrophotometer UV-2450 (Shimadzu) and the UV-visible (vis) spectra has been recorded between wave lengths of 200 nm to 700 nm.

### **XRD Measurement**

X-ray diffraction is a convenient method for determining the mean size of single-crystal nanoparticles. The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 20 min followed by re-dispersion of the pellet of silver nanoparticles into 10 ml of sterile distilled water. After freeze drying of purified silver nanoparticles, the structure of the synthesized silver nanoparticles were investigated analysis using an XRD (RIGAKU-D Machine). The sample was casted on a glass plate and the analysis was made at the voltage of 40 kV and current of 40 mA. The source used was copper  $\text{K}\alpha$  line. Based on the XRD result, the crystalline domain size was calculated from the width of XRD peaks using Scherrer's equation [29] which relates the size of sub-micrometre particles, or crystallites, in a solid to the broadening of a peak in a diffraction pattern.

$$D = \frac{K \lambda}{\beta \cos \theta}$$

$$\text{Where } \beta = \frac{\pi}{180 * FWHM}, \lambda = 1.540598 \text{ \AA}, K\lambda = 0.94 * 1.540598 \text{ \AA} = 1.4482$$

### SEM Analysis of Silver Nano particles

The structural features of the synthesized silver nanoparticles using *Eclipta prostrata* leaf extract were characterized using SEM analysis (PHILIPS-XL-30 SEM ) machine. Thin films of sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting under a tungsten lamp for 10 minutes.

### FTIR Spectrum

Fourier-transform infrared spectral data presented in this work has been recorded using Bruker Tensor 27 model FTIR spectrometer. The spectrum was recorded in mid-IR region of 400-4000 cm<sup>-1</sup> with 16 scan speed, using attenuated total reflectance (ATR) technique.

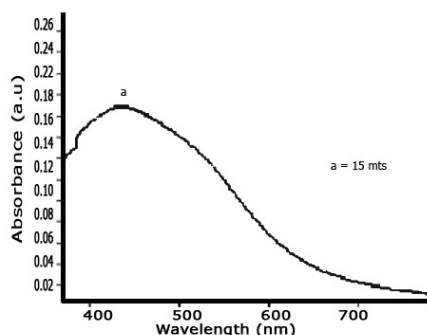
### RESULTS

It was observed that aqueous Ag ions when exposed to *Eclipta prostrata* leaf extract were reduced in solution, thereby leading to the formation of hydrosol. Silver nanoparticles exhibited dark yellowish brown colour in aqueous solution within Two hours (Figure 3) thus indicating the formation of silver nanoparticles.



**Figure 3: Photograph of (a) *Eclipta Prostrata* Leaf Extract, (b) Colloidal Solution of Silver Nanoparticles**

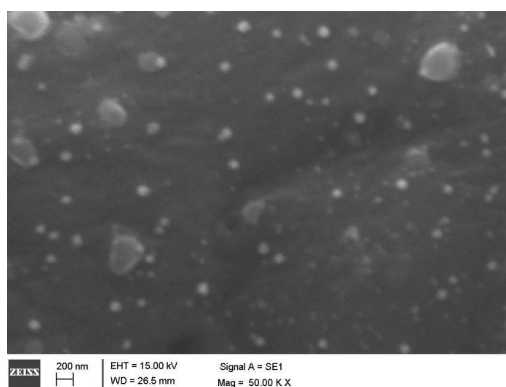
Silver nanoparticles formed in the reaction media has an absorbance peak at 416 nm on UV Vis-spectrophotometer. The peak was broadened (Figure 4).



**Figure 4: UV-Visible Spectra of Ag Nanoparticles**

To study the crystalline nature of the silver nanoparticles, the XRD analysis was undertaken. The dry powder of the silver nanoparticles was used for XRD analysis. The diffraction intensities were recorded from  $10^\circ$  to  $70^\circ$  at  $2\theta$  angles. It revealed three intense peaks in the whole spectrum of  $2\theta$  value ranging from  $10^\circ$  to  $70^\circ$ , corresponding to three diffraction facets of silver. A number of Bragg reflections corresponding to the (111), (200), (220) sets of lattice planes were observed, revealing four peaks [ $\sim 38^\circ$  (111),  $\sim 44^\circ$  (200),  $\sim 64^\circ$  (220)] corresponding to three diffraction facets of silver. A few intense additional and yet unassigned peaks were also noticed in the vicinity of the characteristic peaks of silver ( $\sim 32^\circ$ ,  $\sim 46^\circ$  and  $\sim 76^\circ$ ) which was in agreement with the results obtained by Tripathy *et al.*, [33]. These sharp Bragg peaks might have resulted from some bioorganic compounds/protein(s) present in the *Eclipta* leaf broth. The intensity of the Bragg reflections suggests strong X-ray scattering centres in the crystalline phase and could possibly arise from metalloproteins in the broth. As detailed in the experimental section, the silver nanoparticles after their formation were repeatedly centrifuged and re-dispersed in sterile distilled deionized water as a part of purification, thus ruling out the presence of any free compound/protein that might independently crystallize and give rise to the observed Bragg reflections. The XRD results therefore suggest that the crystallization of the bioorganic phase occurs on the surface of the silver nanoparticles [34]. The particles have size ranging between 11 to 50 nm. An average size of silver nanoparticles synthesized was 34 nm and these were spherical in shape.

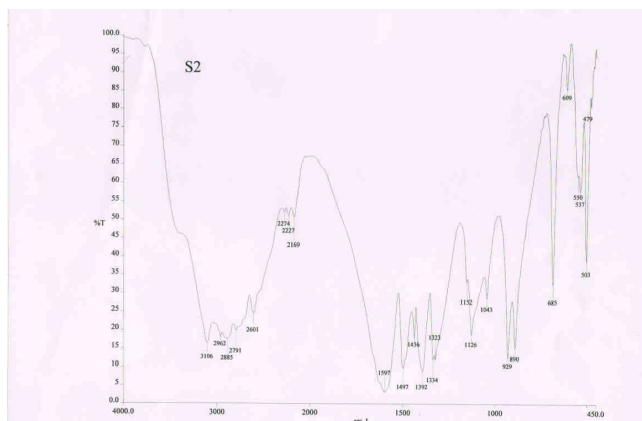
The biosynthesized silver nanoparticles by employing *Eclipta prostrata* leaf extract were further demonstrated and confirmed under scanning electron microscope. The SEM image (Figure 5) depicts high density nanoparticles synthesized by the *Eclipta prostrata* leaf extract and are relatively spherical in shape.



**Figure 5: SEM Image of Silver Nanoparticles Synthesized**

### FTIR Spectroscopy Analysis

FTIR spectroscopy analysis were carried out to identify the biomolecules responsible for capping of the bio-reduced AgNPs synthesized using plant extract. The FTIR spectra of aqueous silver nanoparticles prepared from the *Eclipta prostrata* leaf extract Figure. 6



**Figure 6: FTIR Spectra of Aqueous Silver Nanoparticles**

## DISCUSSIONS

Reduction of silver ions exhibited brown colour in aqueous solution due to surface plasmon vibrations in silver nanoparticles [34]. *Eclipta prostrata* leaf extract was added in the aqueous solution of the silver ion complex, it started to change the colour from watery to yellowish brown due to the reduction of the silver ions (Figure 3). The fundamental mechanism of biosynthesis of silver nanoparticles is not fully understood [35]. Huang et al. (2007) [36] reported that polyol compounds and the water soluble heterocyclic compounds are mainly responsible for the reduction of the silver ions and the stabilization of the nanoparticles respectively. According to [37], proteins are found to be responsible for the reduction of metal ions when plant extracts are used for the synthesis of silver nanoparticles. According to LingaRao and Savitramma[38] secondary metabolites present in plant systems may be responsible for the reduction of  $\text{Ag}^+$  and synthesis of silver nanoparticles. Electrons released during glycolysis led to transformation of silver nitrate to form silver nanoparticles [38].

UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time. Absorption spectra of silver nanoparticles formed in the reaction media after Two hours and it had an absorption peak at 416 nm, broadening of peak indicated that the particles are polydispersed (Figure 4). UV-Vis spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspensions [39].

The FTIR spectrum of Ag nanoparticle is shown in Fig 6. The spectrum of Ag nanoparticles show stretching at (units: wavenumber  $\text{cm}^{-1}$ ) 3106 , 2962, 2885, 2791 , 2601 corresponds to O-H stretching for Carboxylic acids stretching, 2274 corresponds to N-H bond stretching of amines, 2169 corresponds to C-N stretching of the aromatic amino group and C-O stretching of alcohols and ethers respectively, 1126 corresponds to C-O stretch alcohols, 1392, 1436, 1497 corresponds to  $\text{CH}_2$  bend alkenes, 609, 685 and 890 corresponds to C-H bend alkenes. These stretching values indicate that the carbonyl group formed amino acid residues and that these residues “capped” the silver nanoparticles to prevent agglomeration, thereby stabilizing the medium [40]. When the metal nanoparticles form in solution, they must be stabilized against the van der Waals forces of attraction which may otherwise cause coagulation. Physisorbed surfactant and polymers may cause steric or electrostatic barriers or purely electrostatic barriers around the particle surface and may thereby provide stabilization [41]. FTIR peaks that were corresponding to aromatic rings, germinal methyls, and ether linkages indicate the presence of flavones and terpenoids responsible for the stabilization of the AgNPs synthesized by the *Sesuvium portulacastrum* leaf extract [42]. The FTIR spectrum of Ag nanoparticles suggested that Ag nanoparticles were

surrounded by different organic molecules such as terpenoids, alcohols, ketones, aldehydes and carboxylic acids [43].

The average size of silver nanoparticles calculated using Scherrer's formula was 27.4 nm. The silver nanoparticles were spherical in shape. Hence from the XRD pattern it is clear that silver nanoparticles formed using *Eclipta prostrata* leaf extract were essentially crystalline in nature.

## CONCLUSIONS

The biosynthesis of silver nanoparticles using *Eclipta prostrata* leaf extract has been demonstrated. The major conclusions drawn from the above study were the following:

- The shape and size of the nanoparticles produced through bio-reduction by *Eclipta* leaves extract were strongly dependent on the process parameters like *Eclipta* broth concentration, mixing ratio of *Eclipta* extract to AgNO<sub>3</sub> solution, interaction time and pH of the solution.
- The reduction of metal ions through this method lead to the formation of silver nanoparticles of fairly well defined dimensions. In short interaction time of two hours, nanoparticles below 30-nm-size with nearly spherical shape was produced. With increasing interaction time (ageing), the aggregation and shape anisotropy of the particles increased.
- In alkaline pH range, stability of cluster distribution was enhanced decreasing tendency for aggregation of the particles.
- As observed from FT-IR studies and XRD analysis, bio-organic components from *Eclipta* leaf broth acted as probable stabilizer for the silver nanoparticles. The end use of the silver nanoparticles strongly depends on the shape and size of the particles along with the size distribution. In a biological process, fine tuning the process parameters may give product with typical physical characteristics as such a detailed mathematical study of the optical, dielectric, elastic properties of the silver nanoparticles synthesized using the *Eclipta* leaf extract is underway.

Keeping in view the medical applications of the plant *Eclipta prostrata*, this green route of silver nanoparticle synthesis has many advantages such as, ease with which the process can be scaled up, economic viability and eco-friendliness that could help in large scale production of silver nanoparticles.

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